Docket No.: 532232001200
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Jeffrey P. WHITTEN, et al.

Application No.: 10/820,487

Filed: April 7, 2004

For: HETEROCYCLIC SUBSTITUTED 1,4-DIHYDRO-4-OXO-1,8-NAPHTHPYRIDINE ANALOGS

Confirmation No.: 7396

Art Unit: 1625

Examiner: C. Aulakh

DECLARATION OF NICOLE STREINER UNDER 37 C.F.R. § 1.132

MS RCE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, Nicole Streiner, declare as follows:

1. I am a Research Associate III at Cylene Pharmaceuticals, Inc., the owner of the present application. I earned a B.S. in molecular biology from University of Applied Sciences in Mannheim, Germany in 2002, and I have performed molecular biology research for four (4) years.

2. I and another colleague tested three compounds disclosed in the above-identified patent application for biological activity in cell proliferation assays. These assays are described generally in the specification at paragraph 59, page 18. We utilized a cell proliferation assay that monitored the reducing potential of metabolically active proliferating cells. Proliferating cells

EXHIBIT 1

reduce the dye Alamar Blue into a fluorescent product. Non-proliferating cells, such as dying cells, do not reduce the dye into the fluorescent product. Thus, each compound having anti-proliferation activity was identified by detecting a reduced fluorescent signal as compared to cells not treated with the compound.

3. A representative cell-proliferation assay protocol using Alamar Blue dye (stored at 4°C, use 20ul per well) is described below.

96-well plate setup and compound treatment

a. Split and trypsinize cells.

b. Count cells using hemocytometer.

c. Plate 4,000-5,000 cells per well in 100 μl of medium and seed into a 96-well plate according to the following plate layout. Add cell culture medium only to wells B10 to B12. Wells B1 to B9 have cells but no compound added.

	1	2	3	4	5	6	7	8	9	10	11	12	
A	EMPTY												
B	NO COMPOUND ADDED									Medium Only			
C	10nM			100nM			1uM			10uM			QQ58S
D	10nM			100nM			1uM			10uM			Comp1
E	10nM			100nM			1uM			10uM			Comp2
F	10nM			100nM			1uM			10uM			Comp3
G	10nM			100nM			1uM			10uM			Comp4
H	EMPTY												

d. Add 100 μl of 2X drug dilution to each well in a concentration shown in the plate layout above. At the same time, add 100 μl of media into the control wells (wells B10 to B12). Total volume is 200 μl /well.

e. Incubate four (4) days at 37°C, 5% CO_2 in a humidified incubator.

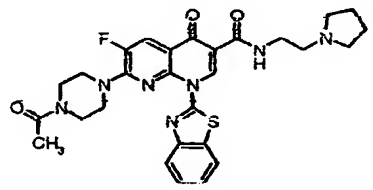
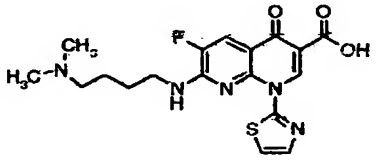
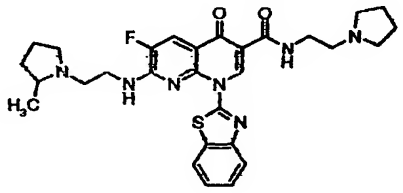
f. Add 20μl Alamar Blue reagent to each well.

g. Incubate for four (4) hours at 37°C, 5% CO_2 in a humidified incubator.

h. Record fluorescence at an excitation wavelength of 544nm and emission wavelength of 590nm using a microplate reader.

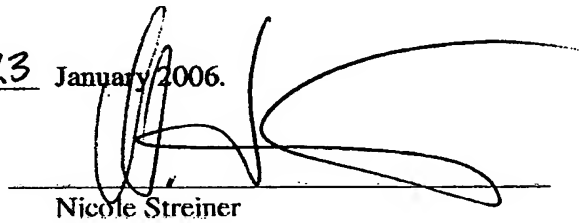
4. In the assays, cells were cultured with a test compound for approximately four days, the dye then was added to the cells and fluorescence of non-reduced dye was detected after approximately four hours. Different types of cells were utilized in the assays. Pancreatic cancer cells (i.e., MiaPaca), colorectal cancer cells (i.e., HCT-116) and cervical cancer cells (i.e., HeLa) were treated in the assays with the compounds and effects on cell proliferation were observed.

5. The structure of each compound, the type of cancer cells treated, and the concentration of each compound that reduced the amount of fluorescent dye by half (IC_{50} concentration in micromolar units) is depicted in the following table. As the data indicate, the compounds reduced proliferation of pancreatic, cervical and colorectal cancer cells.

Compound	Cell Type	IC_{50} (μM)
	Pancreatic cancer	0.02
	Colorectal cancer	0.03
	Cervical cancer	2.5
	Cervical cancer	3.5

6. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements are made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Executed in San Diego, California, on 23 January 2006.



Nicole Streiner